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Actin Polymerization: Riding the Wave

Dispatch

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WAVE/SCAR has long been known to activate the actin-nucleating Arp2/3 complex in a Rac-dependent manner. Recent biochemical and genetic studies have revealed important roles for four WAVE-associated proteins in regulating WAVE function.

Proper execution of a wide variety of actin-dependent processes in eukaryotic cells depends on tight control over the timing and location of actin polymerization. As a nucleator of actin polymerization, the highly conserved Arp2/3 complex plays a central role in the cellular control of actin dynamics [1,2]. The Arp2/3 complex by itself has little effect on actin polymerization, but the complex can be activated by a variety of proteins that couple intracellular or extracellular cues to the control of actin nucleation [1,2]. The most intensively studied Arp2/3 complex activators are members of the WASP/WAVE family, which are regulated in part by Rho-type small GTPases. Purified WASP protein is inactive because of autoinhibition, which is relieved by binding to Cdc42–GTP and phosphatidylinositol 4,5-bisphosphate (PIP_2) [1,2]. In contrast, purified WAVE is fully active [3], but *in vivo* WAVE activity is dependent on Rac–GTP [4].

A mechanism for Rac regulation of WAVE activity was revealed in a seminal recent study [5] showing that WAVE1 protein isolated from bovine brain extracts belongs to multiprotein complex that is inactive at stimulating Arp2/3-dependent actin polymerization *in vitro*. Upon addition of Rac–GTP or the SH2/SH3 domain protein Nck, WAVE1 is released from the complex in an active form (Figure 1). Here we discuss a series of more recent studies [6–9] demonstrating important roles for WAVE complex components in the regulation of actin polymerization *in vivo*. The new results are largely in agreement with the model illustrated in Figure 1, and shed additional light on the regulation of WAVE by these associated proteins.

PIR121, Nap125 and Abi2: Proteins with a Split Personality

In vitro, Rac–GTP and Nck stimulate dissociation of the WAVE complex into two subcomplexes: one comprising WAVE1 and HSPC300; and the other comprising the Rac-associated protein PIR121/Sra-1 [10], the Nck associated protein Nap125 [11] and Abelson tyrosine kinase-interacting protein Abi2 [12] (Figure 1). The fact that WAVE is activated after dissociation from the latter three proteins implies that they are negative regulators of WAVE activity. Recent genetic studies provide evidence of this and for additional roles for PIR121, Nap125 and Abi2 in WAVE regulation *in vivo*.

WAVE was originally discovered in *Dictyostelium* as the product of the *scar* gene: *scar*$^-$ mutant cells display reduced size and motility, as well as reduced F-actin labeling [13]. Recently, a null mutation in the *Dictyostelium* gene encoding PIR121, *pirA*, has been shown to cause a phenotype essentially opposite to that of *scar*$^-$ mutant cells [6]: *pirA*$^-$ mutant cells are abnormally large and display motility defects associated with excess pseudopod formation. Quantitative analysis showed an almost two-fold increase in F-actin content (relative to total actin) in *pirA*$^-$ mutants compared to wild-type cells. Significantly, double mutant *scar*$^-$ *pirA*$^-$ mutant cells have the same phenotype as *scar*$^-$ single mutants, indicating that PIR121 acts on WAVE/SCAR to inhibit its function [6].

Drosophila has a single gene, *scar*, encoding a WAVE homolog. Genetic studies have demonstrated that this gene is critical for a wide variety of actin-dependent processes throughout development [14]. Null mutations in the *kette* gene, which encodes the *Drosophila* homolog of Nap125, cause defects in the actin-dependent processes of neurite growth and glial cell migration; these defects are associated with the formation of disorganized, excess F-actin bundles [15]. Recently, the glial cell migration defect in *kette* mutants was shown to be suppressed in flies hemizygous for the *scar* gene, demonstrating that Kette acts to repress SCAR function [7].

Unexpectedly, other studies have yielded results implying that PIR121/Sra-1, Kette/Nap125 and Abi2 can play positive, rather than inhibitory, roles in WAVE/SCAR-mediated actin polymerization. In cultured Drosophila S2 cells, downregulation by RNA interference (RNAi) of Sra-1, Kette or Abi inhibited lamellipodium formation and led to loss of cortical F-actin, the same phenotype observed after RNAi of SCAR [8,9]. Interestingly, the Sra-1(RNAi), Kette(RNAi) or Abi(RNAi) cells showed a substantial decrease in levels of SCAR protein [8,9]. Similarly, *Dictyostelium* SCAR protein was reduced to barely detectable levels in *pirA*$^-$ mutant cells [6].

These observations reveal an unforeseen function for the WAVE complex in regulating WAVE/SCAR protein levels. Treatment of Abi(RNAi) cells with proteasome inhibitors partially restored SCAR protein levels, suggesting that Abi protects SCAR protein from proteasome-mediated degradation [9]. Degradation of SCAR protein in Sra-1(RNAi), Kette(RNAi) or Abi(RNAi) cells would seem to be sufficient explanation for the similarity of their phenotypes to that of SCAR(RNAi) cells, but additional observations suggest that Abi functions to localize SCAR protein at the cortex as well as to protect it from degradation [9].

How can the results of these RNAi studies be reconciled with the apparently contradictory results from analyses of *Drosophila* and *Dictyostelium* mutants discussed earlier? The answer may lie in the fact that RNAi does not completely eliminate target

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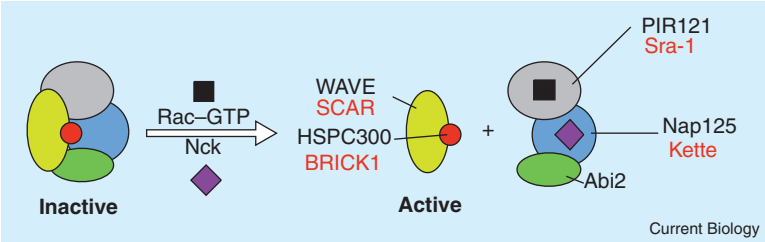

Figure 1. A model for Rac/Nck-mediated activation of WAVE/SCAR.

In the presence of Rac–GTP or Nck, the inactive WAVE complex dissociates into two subcomplexes: one containing WAVE1 and HSPC300, which is active in stimulating Arp2/3-dependent actin polymerization, and one containing PIR121, Nap125 and Abi2 [5]. Hypothetical interactions between Rac–GTP and PIR121, and between Nck and Nap125 are also illustrated, based on the previous identification of PIR121 as a Rac-associated protein [10] and of Nap125 as an Nck-associated protein [11]. Alternative names for PIR121, Nap125, WAVE, and HSPC300 used in this article are shown in red.

proteins, whereas the *kette* and *pirA*$^-$ mutations analyzed are null alleles. If the remaining SCAR protein in Kette(RNAi), Sra-1(RNAi) and Abi(RNAi) cells is present in intact WAVE/SCAR complexes, then these RNAi treatments may reduce F-actin polymerization by reducing levels of SCAR without relieving it from dependence on Rac/Nck-mediated activation. In contrast, the small amounts of SCAR protein remaining in *pirA*$^-$ null mutants may be constitutively active in the complete absence of PIR121 protein, causing excess actin polymerization. Alternatively, differences in the cellular context might explain why reduction or loss of PIR121/Sra-1 or Kette seems to stimulate actin polymerization in some cases and inhibit it in others.

HSPC300: Another Brick in the Wall

Relatively few studies have addressed the function of HSPC300. The observation that this protein remains associated with WAVE following Rac or Nck-mediated dissociation of the WAVE complex does not clearly predict what role HSPC300 might play in WAVE regulation or actin polymerization. RNAi of HSPC300 in *Drosophila* S2 cells produced a mild and variable phenotype resembling that seen in SCAR(RNAi) cells [9]. Earlier work [16], however, showed that, in maize, null mutations in the gene for the HSPC300 homolog BRICK1 cause a failure in the formation of localized cortical F-actin enrichments in dividing and expanding leaf epidermal cells. In the absence of these F-actin enrichments, the epidermal cells fail to undergo specific cell shape changes and to become polarized in preparation for asymmetric cell divisions [16].

These findings suggest that BRICK1 has a positive role in the regulation of a plant Arp2/3 complex activator. The Arp2/3 complex itself is present in plants, and has recently been shown to play an important role in epidermal cell morphogenesis [17]. Genes encoding PIR121 and Nap125 homologs have also been identified in *Arabidopsis*, though they remain to be functionally characterized [17]. But the Arp2/3 complex activator that BRICK1, PIR121 and Nap125 presumably interact with remains to be identified. In any case, results from the analysis of BRICK1 in plants and HSPC300 in *Drosophila* S2 cells suggest that HSPC300 plays an important role in WAVE activation, localization and/or stabilization.

Concluding Perspectives

Genetic analyses of WAVE complex components have come together with biochemical studies to enhance our understanding of how this complex participates in the regulation of WAVE. In particular, these studies have revealed an interesting, dual role for Nap125/Kette, PIR121/Sra-1 and Abi in the regulation of WAVE function. These proteins appear to promote WAVE function by protecting it from degradation and perhaps also helping to localize it correctly, while at the same time inhibiting WAVE's activity. Linking WAVE activation to its degradation in this way might help to ensure that WAVE activation is very tightly coupled to appropriate activating signals.

Many questions remain concerning the functions of WAVE complex components and regulation of the pathway in which they function. One obvious question is why does reduction or loss of PIR121, Nap125, and Abi2 lead in some cases to excess F-actin accumulation and in others to loss of F-actin? If the WAVE complex is activated by Rac–GTP at the plasma membrane, what keeps WAVE there once it dissociates from the complex and Rac? Answers to these questions might reveal new facets of WAVE regulation.

Further questions concern the roles of other proteins implicated in WAVE regulation. One of these is IRSp53, which binds to WAVE2 and enhances its activation of Arp2/3-dependent actin polymerization *in vitro* [18]. Moreover, IRSp53 is implicated in Rac-induced membrane ruffling and can directly link WAVE2 to Rac–GTP *in vitro*, suggesting a role for IRSp53 in Rac regulation of WAVE *in vivo* [18]. A more recently identified WAVE-interacting protein is WRP, a Rac-selective GTPase activating protein. Binding to WAVE has been proposed to direct WRP to Rac, which could attenuate WAVE activation by stimulating Rac's GTPase activity, or could enhance WAVE activation by recycling Rac from the PIR121–Nap125–Abi2 complex. Understanding how the contributions of these WAVE-interacting proteins are integrated to achieve proper regulation of WAVE *in vivo* is an important challenge that lies ahead.

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